=> d ibib abs hitstr l11 1-13

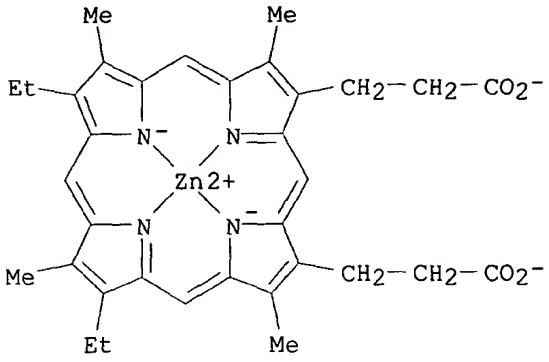
L11 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:699513 HCAPLUS
DOCUMENT NUMBER: 136:33550
TITLE: Binding specificity of the **Porphyromonas gingivalis** heme and hemoglobin receptor HmuR, gingipain K, and gingipain R1 for heme, porphyrins, and metalloporphyrins
AUTHOR(S): Olczak, Teresa; Dixon, Dabney White; Genco, Caroline Attardo
CORPORATE SOURCE: Department of Medicine, Section of Infectious Diseases, Boston University School of Medicine, Boston, MA, 02118, USA
SOURCE: Journal of Bacteriology (2001), 183(19), 5599-5608
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Previous genetic and biochem. studies have confirmed that Hb and hemin utilization in **Porphyromonas gingivalis** is mediated by the outer membrane Hb and heme receptor HmuR, as well as gingipain K (Kgp), a lysine-specific cysteine protease, and gingipain R1 (HRgpA), one of two arginine-specific cysteine proteases. In this study we report on the binding specificity of the recombinant **P. gingivalis** HmuR protein and native gingipains for Hb, hemin, various porphyrins, and metalloporphyrins as assessed by spectrophotometric assays, by affinity chromatog., and by ELISA. Protoporphyrin, mesoporphyrin, deuteroporphyrin, hematoporphyrin, and some of their iron, copper, and zinc derivs. were examd. to evaluate the role of both the central metal ion and the peripheral substituents on binding to recombinant HmuR and sol. gingipains. Scatchard anal. of **hemin binding** to Escherichia coli cells expressing recombinant membrane-assocd. six-His-tagged HmuR yielded a linear plot with a binding affinity of 2.4 .times. 10-5 M. Recombinant E. coli cells bound the iron, copper, and zinc derivs. of protoporphyrin IX (PPIX) with similar affinities, and approx. four times more tightly than PPIX itself, which suggests that the active site of HmuR contains a histidine that binds the metal ion in the porphyrin ring. Furthermore, we found that recombinant HmuR prefers the Et and vinyl side chains of the PPIX mol. to either the larger hydroxyethyl or smaller hydrogen side chains. Kgp and HRgpA were demonstrated to bind various porphyrins and metalloporphyrins with affinities similar to those for hemin, indicating that the binding of Kgp and HRgpA to these porphyrins does not require a metal within the porphyrin ring. We did not detect the binding of RgpB, the arginine-specific cysteine protease that lacks a C-terminal **hemagglutinin** domain, to Hb, porphyrins, or metalloporphyrins. Kgp and HRgpA, but not RgpB, were demonstrated to bind directly to sol. recombinant six-His-tagged HmuR. Several possible mechanisms for the cooperation between outer membrane receptor HmuR and proteases Kgp and HRgpA in hemin and Hb binding and utilization are discussed.

IT **14354-67-7**, Zinc Mesoporphyrin IX **14494-37-2** **14494-42-9**, Copper Mesoporphyrin **15442-64-5**, Zinc protoporphyrin IX **16009-13-5**, **Hemin 18040-04-5**, Iron Mesoporphyrin IX
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**binding** specificity of the **Porphyromonas gingivalis** heme and Hb receptor HmuR, gingipain K, and gingipain R1 for heme, porphyrins, and metalloporphyrins)

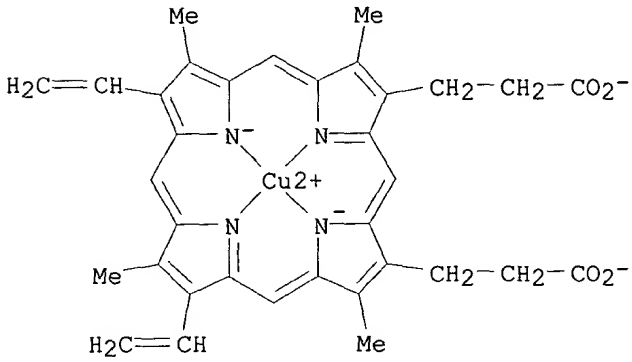
RN 14354-67-7 HCAPLUS

CN Zincate(2-), [7,12-diethyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



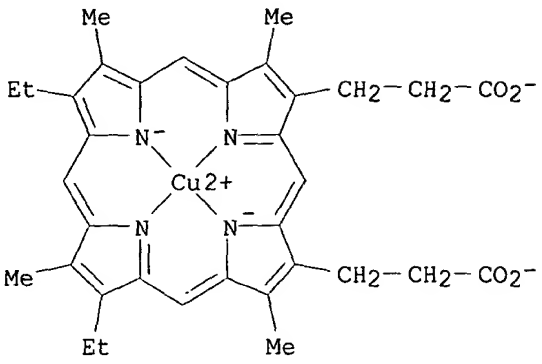
●2 H+

RN 14494-37-2 HCAPLUS
CN Cuprate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



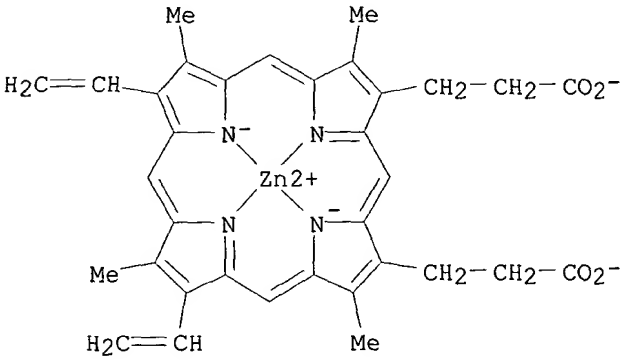
●2 H+

RN 14494-42-9 HCAPLUS
CN Cuprate(2-), [7,12-diethyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



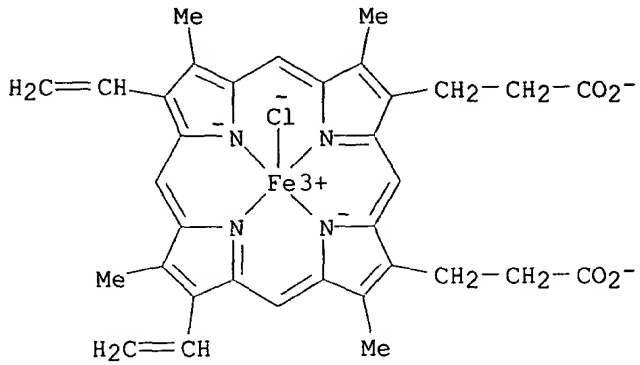
●2 H^+

RN 15442-64-5 HCAPLUS
CN Zincate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



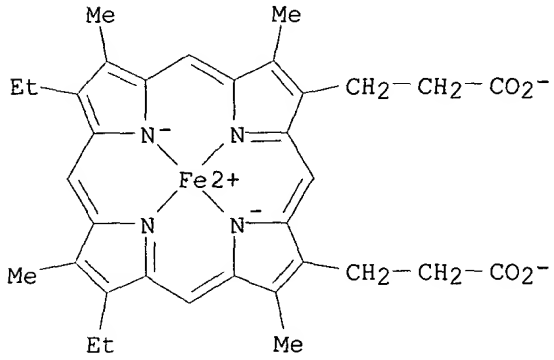
●2 H^+

RN 16009-13-5 HCAPLUS
CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

RN 18040-04-5 HCAPLUS
CN Ferrate(2-), [7,12-diethyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



●2 H^+

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:493373 HCAPLUS
DOCUMENT NUMBER: 135:208048
TITLE: Effects of chlorhexidine digluconate and hydrogen peroxide on **Porphyromonas gingivalis hemin binding** and coaggregation with oral streptococci
AUTHOR(S): Lee, Si Young
CORPORATE SOURCE: Department of Oral Microbiology, College of Dentistry, Kangnung National University, Kangnung, 210-702, S. Korea

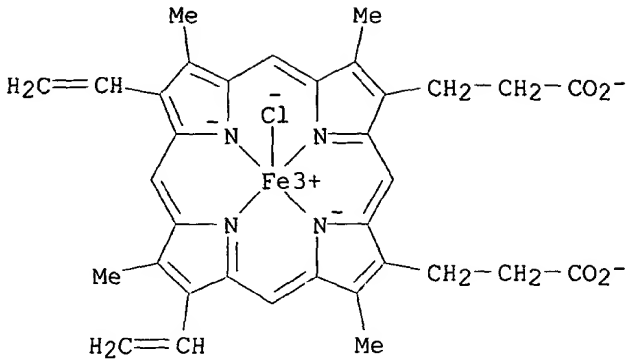
SOURCE: Journal of Oral Science (2001), 43(1), 1-7
CODEN: JORSF3; ISSN: 1343-4934
PUBLISHER: Nihon University School of Dentistry
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Porphyromonas gingivalis**, a gram-neg. anaerobe, is one of the major causative agents of periodontal disease. In this study, the effects of chlorhexidine digluconate and hydrogen peroxide on the **hemin binding** of **P. gingivalis** and coaggregation of this bacterium with oral streptococci were examd. The pretreatment of **P. gingivalis** W50 and 381 with chlorhexidine digluconate and hydrogen peroxide increased the **hemin binding** of these bacteria. The **hemin binding** of **P. gingivalis** was increased by the subminimal inhibitory concn. (MIC) of chlorhexidine digluconate. However, concns. of hydrogen peroxide below the MIC had no effect on the **hemin binding** of **P. gingivalis** W50 and 381. Coaggregation of **P. gingivalis** 381 with Streptococcus oralis ATCC 9811 and Streptococcus gordonii DL1 was diminished by chlorhexidine digluconate. The coaggregation-inhibitory effect was concn.-dependent. Hydrogen peroxide also showed inhibitory effects on the coaggregation of **P. gingivalis** 381 with S. oralis 9811 and S. gordonii DL1 at concns. below that used clin. Concns. of chlorhexidine digluconate below the MIC inhibited coaggregation. However, concns. of hydrogen peroxide below the MIC were not effective in reducing the coaggregation of **P. gingivalis** with oral streptococci. These observations show that chlorhexidine digluconate and hydrogen peroxide could confer variable effects on **P. gingivalis hemin binding** and coaggregation of this bacterium with oral streptococci.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(effects of chlorhexidine digluconate and hydrogen peroxide on **Porphyromonas gingivalis hemin binding** and coaggregation with oral streptococci)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:861515 HCAPLUS
DOCUMENT NUMBER: 134:32939
TITLE: Compositions for prophylaxis and treatment of iron-dependent **Porphyromonas gingivalis** *infections*
INVENTOR(S): Collyer, Charles Andrew; Hunter, Neil; De Carlo, Arthur Anthony, Jr.
PATENT ASSIGNEE(S): University of Sydney, Australia
SOURCE: PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000072875	A1	20001207	WO 2000-AU599	20000526
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1189630	A1	20020327	EP 2000-929110	20000526
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

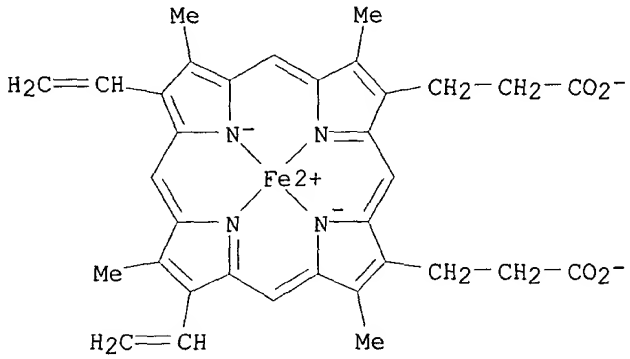
PRIORITY APPLN. INFO.: AU 1999-652 A 19990528
WO 2000-AU599 W 20000526
OTHER SOURCE(S): MARPAT 134:32939
AB The present invention relates generally to a method for the prophylaxis and treatment of infection by microorganisms in biol. environments from

where the microorganisms acquire iron, heme or porphyrin, generally but not exclusively for growth. Particular biol. environments contemplated by the present invention include but are not limited to vascular regions and cavities as well as mucosal membranes in animals including mammals, reptiles, amphibians and fish and in avian species as well as hooves of livestock animals. The method of the present invention involves interrupting, reducing or otherwise antagonizing the interaction between a microbial-derived polypeptide, such as but not limited to a polypeptide having cysteine proteinase activity, and a porphyrin-contg. mol. in such as heme. The present invention further provides agents useful in the prophylaxis and treatment of microbial infection of biol. environments such as vascular regions and cavities including mucosal membranes as well as hooves involving microbial acquisition of iron, heme or porphyrin. Such agents are particularly useful as components in therapeutic compns. Particularly important microbial infections targeted by the present invention involve infections in the oral cavity, nasopharynx, oropharynx, vagina and urethra in mammals such as humans. Other important microbial infections including infections of hooves in livestock animals such as sheep, cattle and goats.

IT **14875-96-8**, Heme
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(acquisition of; compns. for prophylaxis and treatment of iron-dependent **Porphyromonas gingivalis** infections)

RN 14875-96-8 HCAPLUS

CN Ferrate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)



●2 H^+

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:785300 HCAPLUS
DOCUMENT NUMBER: 134:174414
TITLE: Characterization of a novel outer membrane **hemin-binding** protein of **Porphyromonas gingivalis**
AUTHOR(S): Dashper, S. G.; Hendtlass, A.; Slakeski, N.; Jackson,

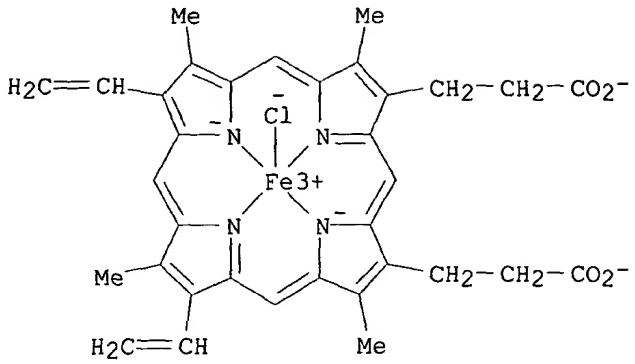
C.; Cross, K. J.; Brownfield, L.; Hamilton, R.; Barr, I.; Reynolds, E. C.
CORPORATE SOURCE: School of Dental Science, The University of Melbourne, Melbourne, 3000, Australia
SOURCE: Journal of Bacteriology (2000), 182(22), 6456-6462
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Porphyromonas gingivalis** is a gram-neg., anaerobic coccobacillus that has been implicated as a major etiol. agent in the development of chronic periodontitis. In this paper, we report the characterization of a protein, IhtB (iron heme transport, formerly designated Pga30), that is an outer membrane **hemin-binding** protein potentially involved in iron assimilation by **P. gingivalis**. IhtB was localized to the cell surface of **P. gingivalis** by Western blot anal. of a Sarkosyl-insol. outer membrane prepn. and by immunocytochem. staining of whole cells using IhtB peptide-specific antisera. The protein, released from the cell surface, was shown to bind to hemin using hemin-agarose. The growth of heme-limited, but not heme-replete, **P. gingivalis** cells was inhibited by preincubation with IhtB peptide-specific antisera. The ihtB gene was located between an open reading frame encoding a putative TonB-linked outer membrane receptor and three open reading frames that have sequence similarity to ATP binding cassette transport system operons in other bacteria. Anal. of the deduced amino acid sequence of IhtB showed significant similarity to the Salmonella typhimurium protein CbiK, a cobalt chelatase that is structurally related to the ATP-independent family of ferrochelatases. Mol. modeling indicated that the IhtB amino acid sequence could be threaded onto the CbiK fold with the IhtB structural model contg. the active-site residues crit. for chelatase activity. These results suggest that IhtB is a peripheral outer membrane chelatase that may remove iron from heme prior to uptake by **P. gingivalis**.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(localization of **Porphyromonas gingivalis hemin-binding** protein IhtB)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:398632 HCAPLUS
DOCUMENT NUMBER: 131:166980
TITLE: Porphyrin-mediated binding to hemoglobin by the **HA2** domain of cysteine proteinases (gingipains) and **hemagglutinins** from the periodontal pathogen **Porphyromonas gingivalis**
AUTHOR(S): DeCarlo, Arthur A.; Paramaesvaran, Mayuri; Yun, Peter L. W.; Collyer, Charles; Hunter, Neil
CORPORATE SOURCE: Department of Periodontics and Department of Oral Biology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA
SOURCE: Journal of Bacteriology (1999), 181(12), 3784-3791
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

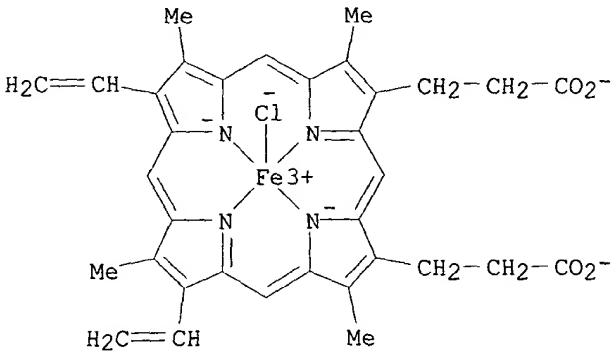
AB Heme binding and uptake are considered fundamental to the growth and virulence of the gram-neg. periodontal pathogen **Porphyromonas gingivalis**. We therefore examd. the potential role of the dominant **P. gingivalis** cysteine proteinases (gingipains) in the acquisition of heme from the environment. A recombinant Hb-binding domain that is conserved between two predominant gingipains (domain **HA2**) demonstrated tight binding to hemin (Kd = 16 nM), and binding was inhibited by iron-free protoporphyrin IX (Ki = 2.5 .mu.M). Hb binding to the gingipains and the recombinant **HA2** (rHA2) domain (Kd = 2.1 nM) was also inhibited by protoporphyrin IX (Ki = 10 .mu.M), demonstrating an essential interaction between the **HA2** domain and the heme moiety in Hb binding. Binding of rHA2 with either hemin, protoporphyrin IX, or hematoporphyrin was abolished by establishing covalent linkage of the protoporphyrin propionic acid side chains to fixed amines, demonstrating specific and directed binding of rHA2 to these protoporphyrins. A monoclonal antibody which recognizes a peptide epitope within the **HA2** domain was employed to demonstrate that **HA2**-assocd. Hb-binding activity was expressed and released by

P. gingivalis cells in a batch culture, in parallel with proteinase activity. Cysteine proteinases from **P. gingivalis** appear to be multidomain proteins with functions for hemagglutination, erythrocyte lysis, proteolysis, and heme binding, as demonstrated here. Detailed understanding of the biochem. pathways for heme acquisition in **P. gingivalis** may allow precise targeting of this crit. metabolic aspect for periodontal disease prevention.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(porphyrin-mediated binding to Hb by the **HA2** domain of cysteine proteinases (gingipains) and **hemagglutinins** from the periodontal pathogen **Porphyromonas gingivalis**)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

REFERENCE COUNT: 68 THERE ARE 68 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1998:57798 HCAPLUS
DOCUMENT NUMBER: 128:202937
TITLE: Hemin regulation of hemoglobin binding by **Porphyromonas gingivalis**
AUTHOR(S): Smalley, John W.; Birss, Andrew J.; McKee, Ailsa S.; Marsh, Philip D.
CORPORATE SOURCE: Department of Clinical Dental Sciences, Unit of Oral Biology, The University of Liverpool, Liverpool, L69 3BX, UK
SOURCE: Current Microbiology (1998), 36(2), 102-106
CODEN: CUMIDD; ISSN: 0343-8651
PUBLISHER: Springer-Verlag New York Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

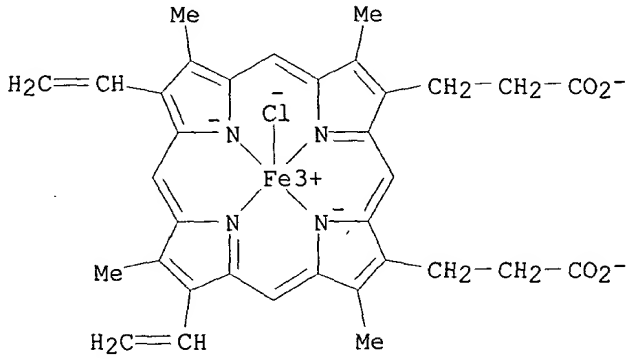
AB Hb binding to chemostat-grown hemin-excess and hemin-limited cells of **Porphyromonas gingivalis** W50, and to cells of the

avirulent, beige-pigmenting variant W50/BE1, was quantified. Hemin-excess W50 bound more Hb than hemin-limited W50, mirroring the **hemin-binding** ability of these cells [Microb Ecol Health Dis 7:9-15, 1994]. In contrast to hemin, Hb binding was not enhanced by sodium dithionite. The Hb-binding capacity of hemin-excess W50/BE1 was below that of hemin-limited W50 and only obsd. under oxidizing conditions. Scatchard anal. revealed similar affinity consts. for hemin-excess and hemin-limited W50, and confirmed a lower binding max. for the latter. Hemin-excess W50/BE1 displayed cooperative binding of Hb. These differences in binding were reflected in the binding of a horse radish peroxidase-conjugated Hb (HHRPO) in a dot-blot assay. However, neither the 32-kDa **hemin-binding** protein, nor its 19-kDa heat-modified form, from either hemin-limited W50 or hemin-excess W50/BE1, bound this conjugate. These data indicate that Hb binding by **P. gingivalis** is hemin-regulated and occurs via a mechanism different from **hemin binding**.

IT **16009-13-5**, Hemin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(hemin regulation of Hb binding by **Porphyromonas gingivalis**)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



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REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:496943 HCAPLUS
DOCUMENT NUMBER: 127:217511
TITLE: The Tla protein of **Porphyromonas gingivalis** W50: a homolog of the RI protease precursor (PrpRI) is an outer membrane receptor required for growth on low levels of hemin
AUTHOR(S): Aduse-Opoku, Joseph; Slaney, Jennifer M.; Rangarajan, Minnie; Muir, Justine; Young, K. Anne; Curtis, Michael A.
CORPORATE SOURCE: MRC Mol. Pathogenesis Group, Dep. Oral Microbiol., St.

Bartholomew's and the Royal London Sch. Medicine and Dentistry, Queen Mary and Westfield College, London, E1 2AA, UK

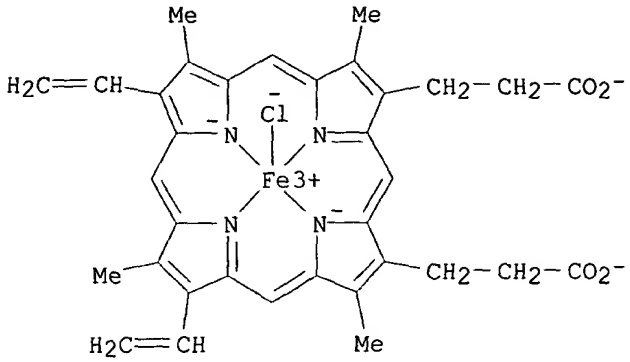
SOURCE: Journal of Bacteriology (1997), 179(15), 4778-4788
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The prpR1 gene of **Porphyromonas gingivalis** W50 encodes the polyprotein precursor (PrpRI) of an extracellular arginine-specific protease. PrpRI is organized into four distinct domains (pro, .alpha., .beta., and .gamma.) and is processed to a heterodimeric protein (RI) which comprises the .alpha. and .beta. components in a noncovalent assocn. The .alpha. component contains the protease active site, whereas the .beta. component appears to have a role in adherence and **hemagglutinination** processes. DNA sequences homologous to the coding region for the RI .beta. component are present at multiple loci on the **P. gingivalis** chromosome and may represent a family of related genes. In this report, we describe the cloning, sequence anal., and characterization of one of these homologous loci isolated in plasmid pJM7. The 6041-bp **P. gingivalis** DNA fragment in pJM7 contains a major open reading frame of 3291 bp with coding potential for a protein with an Mr 118,700. An internal region of the deduced sequence (V304 to N768) shows 98% identity to the .beta. domain of PrpRI, and the recombinant product of pJM7 is immunoreactive with an antibody specific to the RI .beta. component. The N terminus of the deduced sequence has regional similarity to TonB-linked receptors which are frequently involved in periplasmic translocation of hemin, iron, colicins, or vitamin B12 in other bacteria. We have therefore designed this gene tla (TonB-linked adhesin). In contrast to the parent strain, an isogenic mutant of **P. gingivalis** W50 in which the tla was insertionally inactivated was unable to grow in medium contg. low concns. of hemin (<2.5 mg liter-1), and hemin-depleted cells of this mutant failed to respond in an agar diffusion plate assay. These data suggest a role for this gene product in hemin acquisition and utilization. Furthermore, the mutant produced significantly less arginine- and lysine-specific protease activities than the parent strain, indicating that there may be a regulatory relationship between tla and other membranes of this family.

IT **16009-13-5**, Hemin
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Tla protein of **Porphyromonas gingivalis** W50:
homolog of RI protease precursor (PrpRI) is outer membrane receptor required for growth on low levels of hemin)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

L11 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:88883 HCAPLUS
DOCUMENT NUMBER: 126:154917
TITLE: Detection and comparison of specific **hemin binding** by **Porphyromonas gingivalis** and Prevotella intermedia
AUTHOR(S): Tompkins, Geoffrey R.; Wood, Darcy P.; Birchmeier, Kimerberly R.
CORPORATE SOURCE: Dep. Oral Biol., Medical College Georgia, Augusta, GA, 30912-1126, USA
SOURCE: Journal of Bacteriology (1997), 179(3), 620-626
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

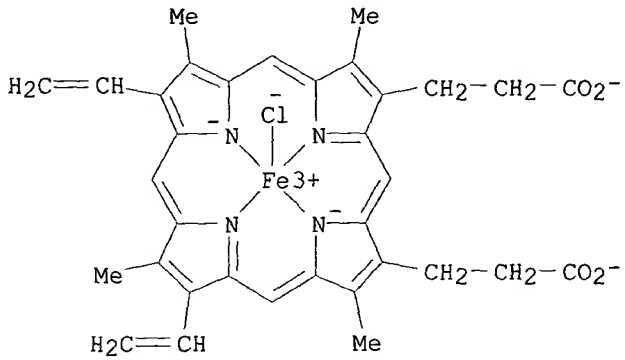
AB A radioligand assay was designed to detect and compare specific **hemin binding** by the periodontal anaerobic black-pigmenting bacteria (BPB) **Porphyromonas gingivalis** and Prevotella intermedia. The assay included physiol. concns. of the **hemin-binding** protein rabbit serum albumin (RSA) to prevent self-aggregation and nonspecific interaction of hemin with cellular components. Under these conditions, heme-starved P. intermedia cells (two strains) expressed a single binding site species (4,100 to 4,600 sites/cell) with a dissocn. const. (Kd) of 1.0 .times. 10-9 M. Heme-starved **P. gingivalis** cells (two strains) expressed two binding site species; the higher-affinity site (1,000 to 1,500 sites/cell)displayed a Kd of between 3.6 .times. 10-11 and 9.6 .times. 10-11 M, whereas the estd. Kd of the lower-affinity site (1.9 .times. 105 to 6.3 .times. 105 sites/cell) ranged between 2.6 .times. 10-7 and 6.5 .times. 10-8 M. Specific binding was greatly diminished in heme-replete cells of either BPB species and was not displayed by iron-replete Escherichia coli cells, which bound as much hemin in the absence of RSA as did P. intermedia. **Hemin binding** by BPB was reduced following treatment with protein-modifying agents (heat, Pronase, and N-bromosuccinimide) and was blocked by protoporphyrin IX and Hb but not by Congo Red. Hemopexin also inhibited bacterial **hemin binding**. These findings indicate that both **P. gingivalis** and P. intermedia express heme-repressible

proteinaceous **hemin-binding** sites with affinities intermediate between those of serum albumin and hemopexin. **P. gingivalis** exhibited a 10-fold-greater specific binding affinity and greater heme storage capacity than did P. intermedia, suggesting that the former would be ecol. advantaged with respect to heme acquisition.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(detection and comparison of specific **hemin binding** by **Porphyromonas gingivalis** and Prevotella intermedia)

RN 16009-13-5 HCAPLUS

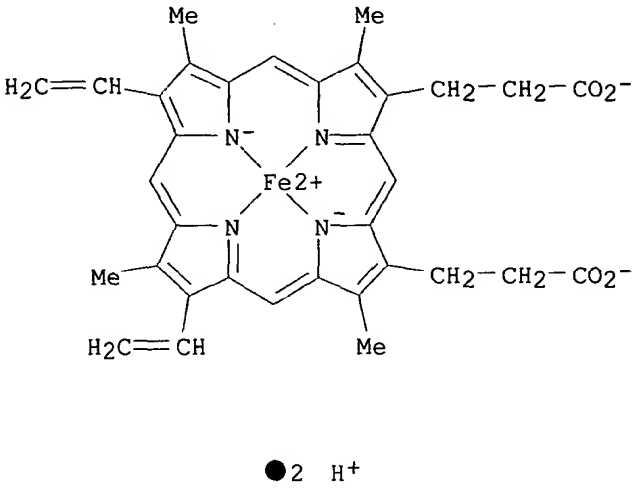
CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



IT **14875-96-8**, Heme
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(repressor; detection and comparison of specific **hemin binding** by **Porphyromonas gingivalis** and Prevotella intermedia)

RN 14875-96-8 HCAPLUS

CN Ferrate(2-), [7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-4-2)- (9CI) (CA INDEX NAME)

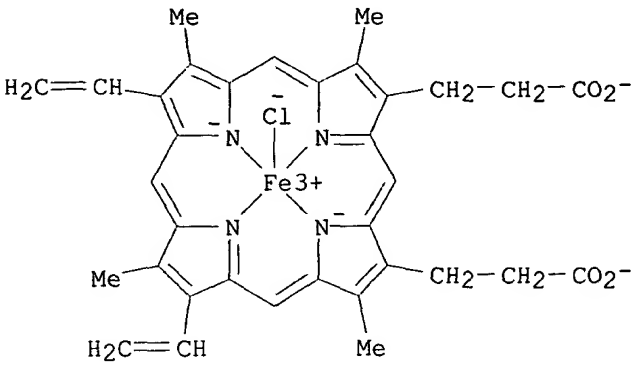


L11 ANSWER 9 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:407617 HCAPLUS
DOCUMENT NUMBER: 125:109788
TITLE: **Hemin binding** as a factor in the virulence of **Porphyromonas gingivalis**
AUTHOR(S): Smalley, John W.; Birss, Andrew J.; McKee, Ailsa S.; Marsh, Philip D.
CORPORATE SOURCE: Unit of Oral Biology, Edwards Building, Department of Clinical Dental Sciences, University of Liverpool, Liverpool, L69 3BX, UK
SOURCE: FEMS Microbiology Letters (1996), 141(1), 65-70
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Hemin (iron protoporphyrin IX) is an essential growth factor for the periodontal pathogen, **Porphyromonas gingivalis**. Iron protoporphyrin IX (IPP IX) binding to the avirulent **P. gingivalis** beige variant (W50/BE1) and the black-pigmenting parent wild-type strain W50 was quantified. W50/BE1 grown in a chemostat under hemin excess-bound IPP IX under both oxidizing and reducing conditions but with both lower capacity and avidity than either the hemin-limited-and hemin-excess-grown parent strain W50. Rosenthal plots for W50/BE1 indicated cooperative binding. W50/BE1 cells expressed a 32 kDa outer membrane **hemin-binding** protein when grown under conditions of hemin excess, and this strain might serve as a useful source from which to isolate this protein. The reduced IPP IX binding ability of W50/BE1 may be the rate-limiting factor for heme uptake and explain the reduced virulence and slower rate of pigmentation of this strain.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**hemin binding** ability in virulence of **Porphyromonas gingivalis**)
RN 16009-13-5 HCAPLUS
CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-,

dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

L11 ANSWER 10 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:328005 HCAPLUS
DOCUMENT NUMBER: 125:8278
TITLE: Hemin-induced modifications of the antigenicity and **hemin-binding** capacity of **Porphyromonas gingivalis** lipopolysaccharide
AUTHOR(S): Cutler, Christopher W.; Eke, Paul I.; Genco, Caroline A.; Van Dyke, Thomas E.; Arnold, Roland R.
CORPORATE SOURCE: Department Biomedical Sciences and Periodontics, Baylor College Dentistry, Dallax, TX, USA
SOURCE: Infection and Immunity (1996), 64(6), 2282-2287
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

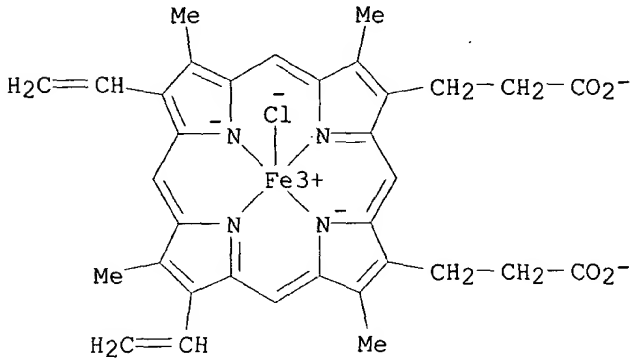
AB Previous studies have shown that the phys., biochem., and antigenic properties of the bacterial outer membrane are profoundly influenced by the growth environment. In the present study, the effects of growth in hemin-replete (H+) and hemin-depleted (H-) media on the lipopolysaccharide (LPS) of the oral pathogen **Porphyromonas gingivalis** were investigated. Our studies show that LPS from **P. gingivalis** cultured in H+ media (H+LPS) expressed addnl. low-mol.-mass antigens, as detd. by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot (immunoblot) anal. Particularly evident was a 26-kDa antigen (26 LPSC) that was lost from the LPS upon transfer of **P. gingivalis** into H- media. The loss of the 26 LPSC was accompanied by a marked redn. in the **hemin-binding** capacity of the LPS. The 26 LPSC was refractory to Coomassie blue staining and proteinase K digestion H+ LPS from strain W50/BE1, a nonpigmented pleiotropic strain, lacked the 26 LPSC and did not bind hemin. Polyclonal antiserum raised to whole-cell antigens of **P. gingivalis** A7436, W83, and HG405 grown in H+ media, but not in H- media, from humans with (n = 24) or without (n = 25) periodontitis to the 26 LPSC and other H+LPS determinants were analyzed by Western blot. Overall, 75% of adult periodontitis patient sera reacted

with multiple bands in the H+LPS stepladder, particularly in the range of 14 to 27 kDa. In contrast, only 20% of control sera reacted faintly with H+LPS bands in the range 27 to 34 kDa. The 26 LPSC was recognized by over 40% of sera from adult patients with periodontitis and none of the healthy control sera. These results suggest that the antigenicity and **hemin-binding** properties of **P. gingivalis** LPS can be modified by growth in H+ media.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(hemin-induced modifications of the antigenicity and **hemin-binding** capacity of **Porphyromonas gingivalis** lipopolysaccharide)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



●2 H^+

L11 ANSWER 11 OF 13 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1995:659111 HCAPLUS

DOCUMENT NUMBER: 123:51998

TITLE: Characterization of a Tn4351-generated hemin uptake mutant of **Porphyromonas gingivalis** : evidence for the coordinate regulation of virulence factors by hemin

AUTHOR(S): Genco, C. A.; Simpson, W.; Forng, R. Y.; Egal, M.; Odusanya, B. M.

CORPORATE SOURCE: Dep. Microbiol., Morehouse Sch. Med., Atlanta, GA, 30310-1495, USA

SOURCE: Infection and Immunity (1995), 63(7), 2459-66
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

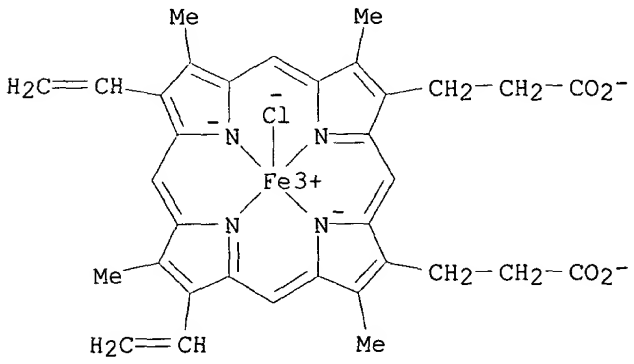
AB The ability of **P. gingivalis** to acquire Fe in the Fe-limited environment of the host is crucial to the colonization of this organism. The isolation and characterization of a transpositional insertion mutant of **P. gingivalis** A7436 (designated

MSM-3) which is defective in the utilization and transport of hemin are reported here. **P. gingivalis** MSM-3 was selected on the basis of its nonpigmented phenotype on anaerobic blood agar following mutagenesis with the Bacteroides fragilis transposon Tn4351. **P. gingivalis** MSM-3 grew poorly when supplied with hemin as a sole source of Fe; however, growth was obsd. with Hb or inorg. Fe. **P. gingivalis** MSM-3 grown in either hemin-replete or hemin-depleted conditions bound and transported less [14C]hemin or [59Fe]hemin than did the parent strain. At 4 h, **P. gingivalis** MSM-3 grown in hemin-replete conditions transported only 10,000 pmol hemin/mg protein, or 14% of the amt. transported by **P. gingivalis** A7436. Unlike **P. gingivalis** A7436, **hemin binding** and transport by gingivalis MSM-3 were not tightly regulated by hemin or Fe. Examn. of **P. gingivalis** MSM-3 cultures by electron microscopy revealed an overprodn. of membrane vesicles, and detn. of the dry wt. of purified vesicles indicated that **P. gingivalis** MSM-3 produced twice as much membrane vesicles as did strain A7436. Extracellular vesicles isolated from **P. gingivalis** MSM-3 also expressed more hemolytic and trypsin-like protease activities than the parent strain. When inoculated into s.c. chambers implanted in mice, **P. gingivalis** MSM-3 was highly infectious and more invasive than the parent strain, as indicated by secondary lesion formation and death. Taken together, these results indicate that the decreased transport of hemin by **P. gingivalis** MSM-3 results in the increased expression of several virulence factors which may be coordinately regulated by hemin.

IT **16009-13-5**, Hemin
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(decreased transport of hemin by **P. gingivalis** mutant results in the increased expression of several virulence factors)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



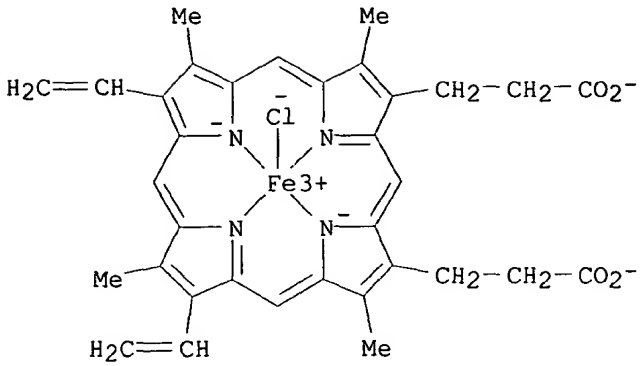
●2 H^+

L11 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1994:600583 HCAPLUS
DOCUMENT NUMBER: 121:200583
TITLE: Binding and accumulation of hemin in **Porphyromonas gingivalis** are induced by hemin
AUTHOR(S): Genco, Caroline Attardo; Odusanya, Basil Michael; Brown, Gene
CORPORATE SOURCE: Dep. Microbiology Immunology, Morehouse Sch. Med., Atlanta, GA, 30310, USA
SOURCE: Infection and Immunity (1994), 62(7), 2885-92
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although hemin is an essential nutrient for the black-pigmented oral bacterium **Porphyromonas gingivalis**, the mechanisms involved in **hemin binding** and uptake are poorly defined. In this study, we have examd. the binding of hemin and Congo red (CR) to **P. gingivalis** whole cells and have defined the conditions for maximal binding. Addnl., the accumulation of hemin by **P. gingivalis** under growing conditions has been characterized. **P. gingivalis** A7436 was grown under hemin- or iron-deplete conditions (basal medium [BM] or Schaedler broth with dipyridyl [SBD]) or under hemin- or iron-replete conditions (BM with hemin [BMH] or Schaedler broth [SB]), and hemin and CR binding were assessed spectrophotometrically. Binding of hemin by **P. gingivalis** whole cells was rapid and was obsd. in samples obtained from cells grown under hemin- and iron-replete and hemin-deplete conditions but was not obsd. in cells grown under iron limitation. We also found that **P. gingivalis** whole cells bound more hemin when grown in BMH or SB than cells grown in BM or SBD. Binding of CR by **P. gingivalis** A7436 was also enhanced when cells were grown in the presence of hemin or when cells were incubated with hemin prior to CR binding. **Hemin binding** and accumulation were also assessed using [14C]hemin and [59Fe]hemin under growing conditions. Both [14C]hemin and [59Fe]hemin were accumulated by **P. gingivalis**, indicating that iron and the porphyrin ring were taken into the cell. Binding and accumulation of hemin under growing conditions were also induced by growth of **P. gingivalis** in hemin-replete media. Hemin accumulation was inhibited by the addn. of KCN to **P. gingivalis** cultures, indicating that active transport was required for hemin uptake. [14C]**hemin binding** and accumulation were also inhibited by the addn. of either cold hemin or protoporphyrin IX. Taken together, these results indicate that **P. gingivalis** transports the entire hemin moiety into the cell and that the binding and accumulation of hemin are induced by growth of cultures in the presence of hemin.

IT **16009-13-5**, Hemin
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**hemin binding** and accumulation by **Porphyromonas gingivalis** are induced by hemin)

RN 16009-13-5 HCAPLUS
CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



L11 ANSWER 13 OF 13 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1994:27211 HCAPLUS
DOCUMENT NUMBER: 120:27211
TITLE: Hemin uptake in **Porphyromonas gingivalis**: Omp26 is a **hemin-binding** surface protein
AUTHOR(S): Bramanti, Thomas E.; Holt, Stanley C.
CORPORATE SOURCE: Health Sci. Cent., Univ. Texas, San Antonio, TX, 78284-7894, USA
SOURCE: Journal of Bacteriology (1993), 175(22), 7413-20
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

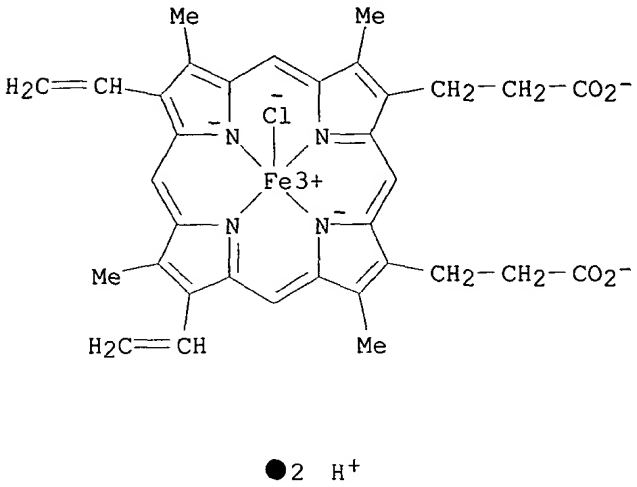
AB A 26-kDa outer membrane protein (Omp26) has been proposed to play a role in hemin acquisition by **Porphyromonas gingivalis**. [55Fe]hemin uptake was studied in **P. gingivalis** grown under conditions of hemin starvation (Omp26 expressed on the outer membrane surface) and hemin excess (Omp26 not expressed on surface). [55Fe]hemin within 3 min. **P. gingivalis** grown under hemin-starved conditions or treated with the iron chelator 2,2'-bipyridyl to induce an iron stress took up six times more [55Fe] hemin than hemin-excess-grown cells. Polyclonal monospecific anti-Omp26 antibody added to hemin-starved cells inhibited [55Fe]hemin uptake by more than 50%, whereas preimmune serum had no effect. [55Fe]hemin uptake in hemin-starved **P. gingivalis** was inhibited (36 to 67%) in the presence of equimolar amts. of unlabeled hemin, protoporphyrin IX, zinc protoporphyrin, and Congo red dye but was not inhibited in the presence of non-hemin-contg. iron sources. Heat shock treatment (45.degree.) of hemin-excess-grown **P. gingivalis** (which causes translocation of Omp26 to the surface) increased [55Fe]hemin uptake by 3-fold after 3 min in comparison with cells grown at 37.degree.. However, no [55Fe]hemin uptake beyond 3 min was obsd. in either hemin-excess-grown or hemin-starved cells exposed to heat shock. In expts. using heterobifunctional cross-linker anal., hemin and selected porphyrins were cross-linked to Omp26 in hemin-starved **P. gingivalis**, but no crosslinking was seen with hemin-excess-grown cells. However, crosslinking of hemin to Omp26 was obsd. after heat shock treatment of hemin-excess-grown cells. Finally, anti-Omp26 antibody inhibited crosslinking of hemin to Omp26. The findings indicate that

hemin binding and transport into the **P. gingivalis** cell is mediated by Omp26.

IT **16009-13-5**, Hemin
RL: PROC (Process)
(uptake of, by **Porphyromonas gingivalis**, Omp26 protein in)

RN 16009-13-5 HCAPLUS

CN Ferrate(2-), chloro[7,12-diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoato(4-)-.kappa.N21,.kappa.N22,.kappa.N23,.kappa.N24]-, dihydrogen, (SP-5-13)- (9CI) (CA INDEX NAME)



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L13 ANSWER 1 OF 32 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2003167340 MEDLINE
DOCUMENT NUMBER: 22557895 PubMed ID: 12670977
TITLE: Porphyrin-mediated cell surface heme capture from hemoglobin by **Porphyromonas gingivalis.**
AUTHOR: Paramaesvaran Mayuri; Nguyen Ky-Anh; Caldon Elizabeth; McDonald James A; Najdi Sherean; Gonzaga Graciel; Langley David B; DeCarlo Arthur; Crossley Maxwell J; Hunter Neil; Collyer Charles A
CORPORATE SOURCE: Institute of Dental Research, Centre for Oral Health, Westmead Hospital, Wentworthville, Sydney NSW 2145, Australia.
SOURCE: JOURNAL OF BACTERIOLOGY, (2003 Apr) 185 (8) 2528-37. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20030416
Last Updated on STN: 20030513
Entered Medline: 20030509

AB The porphyrin requirements for growth recovery of **Porphyromonas gingivalis** in heme-depleted cultures are investigated. In addition to physiologically relevant sources of heme, growth recovery is stimulated by a number of noniron porphyrins. These data demonstrate that, as for Haemophilus influenzae, reliance on captured iron and on exogenous porphyrin is manifest as an absolute growth requirement for heme. A number of outer membrane proteins including some gingipains contain the hemoglobin receptor (**HA2**) domain. In cell surface extracts, polypeptides derived from **HA2**-containing proteins predominated in hemoglobin binding. The in vitro porphyrin-binding properties of a recombinant **HA2** domain were investigated and found to be iron independent. Porphyrins that differ from protoporphyrin IX in only the vinyl aspect of the tetrapyrrole ring show comparable effects in competing with hemoglobin for **HA2** and facilitate growth recovery. For some porphyrins which differ from protoporphyrin IX at both propionic acid side chains, the modification is detrimental in both these assays. Correlations of porphyrin competition and growth recovery imply that the **HA2** domain acts as a high-affinity hemophore at the cell surface to capture porphyrin from hemoglobin. While some proteins involved with heme capture bind directly to the iron center, the **HA2** domain of **P. gingivalis** recognizes heme by a mechanism that is solely porphyrin mediated.

L13 ANSWER 2 OF 32 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002741175 MEDLINE
DOCUMENT NUMBER: 22392639 PubMed ID: 12504090
TITLE: A 35-kDa co-aggregation factor is a **hemin binding** protein in **Porphyromonas gingivalis.**
AUTHOR: Shibata Yasuko; Hiratsuka Koichi; Hayakawa Mitsuo; Shiroza Teruaki; Takiguchi Hisashi; Nagatsuka Yasuko; Abiko Yoshimitsu
CORPORATE SOURCE: Department of Biochemistry, Nihon University School of Dentistry at Matsudo, Chiba 271-8587, Japan.

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2003 Jan 10) 300 (2) 351-6.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021231
Last Updated on STN: 20030226
Entered Medline: 20030225

AB It has been known that **Porphyromonas gingivalis** has an obligate requirement for hemin or selected heme- or Fe-containing compounds for its growth. In addition, the influence of hemin on the expression of several putative virulence factors produced by this bacterium has also been recently documented; however, the mechanisms involved in hemin uptake are poorly defined. We succeeded in cloning the gene coding for the 35-kDa protein, which was specifically expressed in **P. gingivalis** and seemed to confer colonizing activities. Recently, we have constructed the **P. gingivalis** 381 mutant defective in the 35-kDa protein by insertion mutagenesis. The beige mutant exhibited little co-aggregation and the virulence was also decreased. Based on these results and homology search analysis, we focused on assessing the **hemin bindings** and found the heme regulatory motif (HRM) as a hemin direct binding site. The 35-kDa protein did possess the binding ability of selected protoporphyrins involving the hemin. These results demonstrated that 35-kDa protein is one of the **hemin binding** proteins in **P. gingivalis** and suggested that **hemin binding** ability of 35-kDa protein is important for the expression of virulence in **P. gingivalis**.

L13 ANSWER 3 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2003067529 EMBASE

TITLE: Human monoclonal antibody inhibits **porphyromonas gingivalis hemagglutinin** activity.

AUTHOR: Kaizuka K.; Hosogi Y.; Hayakawa M.; Shibata Y.; Abiko Y.
CORPORATE SOURCE: Dr. Y. Abiko, Department of Biochemistry, Nihon Univ. School Dentistry Matsudo, 2-870-1, Sakaecho-Nishi, Matsudo, Chiba 271-8587, Japan. yabiko@mascat.nihon-u.ac.jp

SOURCE: Journal of Periodontology, (1 Jan 2003) 74/1 (38-43).
Refs: 27
ISSN: 0022-3492 CODEN: JOPRAJ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
011 Otorhinolaryngology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Background: **Porphyromonas gingivalis** has been implicated as an important pathogen in the development of chronic periodontitis, and its colonization of subgingival sites is critical in the pathogenic process. One potential virulence factor, **hemagglutinin**, may mediate bacteria attachment onto and penetration into host cells, as well as agglutinate and lyse erythrocytes to intake heme, an absolute requirement for growth. We previously cloned the gene encoding the 130 kDa **hemagglutinin** domain (130k HMGD)

and identified its functional domain. The construction of a human monoclonal antibody that is capable of inhibiting the hemagglutinating ability is significant and important toward the development of passive immunotherapy. Methods: Human lymphocytes isolated from a donor, who had high antibody titer against the recombinant 130k HMGD (r130k HMGD), were immortalized by Epstein-Barr virus, and specific antibody-producing B cells were established by panning using the r130k HMGD. Results: The constructed HuMAb-HMGD1, IgG subclass, recognized the r130k HMGD as well as the 43 and 49 kDa major bands in **P. gingivalis** cells and vesicles. The HuMAb-HMGD1 significantly inhibited hemagglutinating activity of **P. gingivalis** vesicles in a dose-dependent manner. Furthermore, the HuMAb-HMGD1 recognized the synthetic peptide, EGSNEFAPVQNLTGSSVG, which contains the functional domain of 130k HMGD. Conclusion: The newly constructed HuMAb-HMGD1 may prove to be useful for the development of passive immunization against periodontal diseases caused by **P. gingivalis** infection, pending the results of fertility study in disease mode.

L13 ANSWER 4 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2003:167430 BIOSIS
DOCUMENT NUMBER: PREV200300167430
TITLE: Isolation of hemin related gene (HemII) for the **hemin binding** protein from **Porphyromonas gingivalis.**
AUTHOR(S): Kim, S. (1)
CORPORATE SOURCE: (1) College of Dentistry, Pusan National University, Pusan, South Korea South Korea
SOURCE: Molecular Biology of the Cell, (Nov. 2002, 2002) Vol. 13, No. Supplement, pp. 517a. print.
Meeting Info.: 42nd Annual Meeting of the American Society for Cell Biology San Francisco, CA, USA December 14-18, 2002 American Society for Cell Biology
. ISSN: 1059-1524.
DOCUMENT TYPE: Conference
LANGUAGE: English

L13 ANSWER 5 OF 32 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001495613 MEDLINE
DOCUMENT NUMBER: 21429233 PubMed ID: 11544222
TITLE: Binding specificity of the **Porphyromonas gingivalis** heme and hemoglobin receptor HmuR, gingipain K, and gingipain R1 for heme, porphyrins, and metalloporphyrins.
AUTHOR: Olczak T; Dixon D W; Genco C A
CORPORATE SOURCE: Department of Medicine, Section of Infectious Diseases, Boston University School of Medicine, Boston, Massachusetts 02118, USA.
CONTRACT NUMBER: AI45883 (NIAID)
DE09161 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (2001 Oct) 183 (19) 5599-608.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20011015
Entered Medline: 20011011
AB Previous genetic and biochemical studies have confirmed that hemoglobin

and hemin utilization in **Porphyromonas gingivalis** is mediated by the outer membrane hemoglobin and heme receptor HmuR, as well as gingipain K (Kgp), a lysine-specific cysteine protease, and gingipain R1 (HRgpA), one of two arginine-specific cysteine proteases. In this study we report on the binding specificity of the recombinant **P. gingivalis** HmuR protein and native gingipains for hemoglobin, hemin, various porphyrins, and metalloporphyrins as assessed by spectrophotometric assays, by affinity chromatography, and by enzyme-linked immunosorbent assay. Protoporphyrin, mesoporphyrin, deuteroporphyrin, hematoporphyrin, and some of their iron, copper, and zinc derivatives were examined to evaluate the role of both the central metal ion and the peripheral substituents on binding to recombinant HmuR and soluble gingipains. Scatchard analysis of **hemin binding** to Escherichia coli cells expressing recombinant membrane-associated six-His-tagged HmuR yielded a linear plot with a binding affinity of 2.4 x 10(-5) M. Recombinant E. coli cells bound the iron, copper, and zinc derivatives of protoporphyrin IX (PPIX) with similar affinities, and approximately four times more tightly than PPIX itself, which suggests that the active site of HmuR contains a histidine that binds the metal ion in the porphyrin ring. Furthermore, we found that recombinant HmuR prefers the ethyl and vinyl side chains of the PPIX molecule to either the larger hydroxyethyl or smaller hydrogen side chains. Kgp and HRgpA were demonstrated to bind various porphyrins and metalloporphyrins with affinities similar to those for hemin, indicating that the binding of Kgp and HRgpA to these porphyrins does not require a metal within the porphyrin ring. We did not detect the binding of RgpB, the arginine-specific cysteine protease that lacks a C-terminal **hemagglutinin** domain, to hemoglobin, porphyrins, or metalloporphyrins. Kgp and HRgpA, but not RgpB, were demonstrated to bind directly to soluble recombinant six-His-tagged HmuR. Several possible mechanisms for the cooperation between outer membrane receptor HmuR and proteases Kgp and HRgpA in hemin and hemoglobin binding and utilization are discussed.

L13 ANSWER 6 OF 32 MEDLINE
ACCESSION NUMBER: 2001533471 MEDLINE
DOCUMENT NUMBER: 21464034 PubMed ID: 11579579
TITLE: Pathophysiological roles of two types of gingipains in periodontal diseases.
AUTHOR: Yamamoto K; Baba A; Okamoto K; Kadowaki Tkyama@dent.kyushu-u.ac.jp
SOURCE: TANPAKUSHITSU KAKUSAN KOSO. PROTEIN, NUCLEIC ACID, ENZYME, (2001 Aug) 46 (11 Suppl) 1781-8. Ref: 37
Journal code: 0413762. ISSN: 0039-9450.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011003
Last Updated on STN: 20020122
Entered Medline: 20011212

L13 ANSWER 7 OF 32 MEDLINE
ACCESSION NUMBER: 2001311254 MEDLINE
DOCUMENT NUMBER: 21276940 PubMed ID: 11383630
TITLE: Effects of chlorhexidine digluconate and hydrogen peroxide on **Porphyromonas gingivalis**

hemin binding and coaggregation with oral streptococci.

AUTHOR: Lee S Y
CORPORATE SOURCE: Department of Oral Microbiology, College of Dentistry, Kangnung National University, Korea.. siyoung@knusun.kangnung.ac.kr

SOURCE: JOURNAL OF ORAL SCIENCE, (2001 Mar) 43 (1) 1-7.
Journal code: 9808942. ISSN: 1343-4934.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20010813
Entered Medline: 20010809

AB **Porphyromonas gingivalis**, a gram-negative anaerobe, is one of the major causative agents of periodontal disease. In this study, the effects of chlorhexidine digluconate and hydrogen peroxide on the **hemin binding** of **P. gingivalis** and coaggregation of this bacterium with oral streptococci were examined. The pretreatment of **P. gingivalis** W50 and 381 with chlorhexidine digluconate and hydrogen peroxide increased the **hemin binding** of these bacteria. The **hemin binding** of **P. gingivalis** was increased by the subminimal inhibitory concentration (MIC) of chlorhexidine digluconate. However, concentrations of hydrogen peroxide below the MIC had no effect on the **hemin binding** of **P. gingivalis** W50 and 381. Coaggregation of **P. gingivalis** 381 with Streptococcus oralis ATCC 9811 and Streptococcus gordonii DL1 was diminished by chlorhexidine digluconate. The coaggregation-inhibitory effect was concentration-dependent. Hydrogen peroxide also showed inhibitory effects on the coaggregation of **P. gingivalis** 381 with S. oralis 9811 and S. gordonii DL1 at concentrations below that used clinically. Concentrations of chlorhexidine digluconate below the MIC inhibited coaggregation. However, concentrations of hydrogen peroxide below the MIC were not effective in reducing the coaggregation of **P. gingivalis** with oral streptococci. These observations show that chlorhexidine digluconate and hydrogen peroxide could confer variable effects on **P. gingivalis hemin binding** and coaggregation of this bacterium with oral streptococci.

L13 ANSWER 8 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000388503 EMBASE
TITLE: Characterization of a novel outer membrane **hemin-binding** protein of **Porphyromonas gingivalis**.

AUTHOR: Dashper S.G.; Hendtlass A.; Slakeski N.; Jackson C.; Cross K.J.; Brownfield L.; Hamilton R.; Barr I.; Reynolds E.C.
CORPORATE SOURCE: E.C. Reynolds, School of Dental Science, University of Melbourne, 711 Elizabeth St., Melbourne, Vic. 3000, Australia. e.reynolds@dent.unimelb.edu.au

SOURCE: Journal of Bacteriology, (2000) 182/22 (6456-6462).
Refs: 51
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

SUMMARY LANGUAGE: English

AB **Porphyromonas gingivalis** is a gram-negative, anaerobic coccobacillus that has been implicated as a major etiological agent in the development of chronic periodontitis. In this paper, we report the characterization of a protein, IhtB (iron heme transport; formerly designated Pga30), that is an outer membrane **hemin-binding** protein potentially involved in iron assimilation by **P. gingivalis**. IhtB was localized to the cell surface of **P. gingivalis** by Western blot analysis of a Sarkosyl-insoluble outer membrane preparation and by immunocytochemical staining of whole cells using IhtB peptide-specific antisera. The protein, released from the cell surface, was shown to bind to hemin using hemin-agarose. The growth of heme-limited, but not heme-replete, **P. gingivalis** cells was inhibited by preincubation with IhtB peptide-specific antisera. The ihtB gene was located between an open reading frame encoding a putative TonB-linked outer membrane receptor and three open reading frames that have sequence similarity to ATP binding cassette transport system operons in other bacteria. Analysis of the deduced amino acid sequence of IhtB showed significant similarity to the Salmonella typhimurium protein CbiK, a cobalt chelatase that is structurally related to the ATP-independent family of ferrochelatases. Molecular modeling indicated that the IhtB amino acid sequence could be threaded onto the CbiK fold with the IhtB structural model containing the active-site residues critical for chelatase activity. These results suggest that IhtB is a peripheral outer membrane chelatase that may remove iron from heme prior to uptake by **P. gingivalis**.

L13 ANSWER 9 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999214123 EMBASE
TITLE: Genetic analyses of proteolysis, hemoglobin binding, and hemagglutination of **Porphyromonas gingivalis**: Construction of mutants with a combination of rgpA, rgpB, kgp, and hagA.
AUTHOR: Shi Y.; Ratnayake D.B.; Okamoto K.; Abe N.; Yamamoto K.; Nakayama K.
CORPORATE SOURCE: K. Nakayama, Dept. of Microbiology, Faculty of Dentistry, Kyushu University, 3-1-1 Higashi-ku, Fukuoka 812-8582, Japan. knak@dent.kyushu-u.ac.jp
SOURCE: Journal of Biological Chemistry, (18 Jun 1999) 274/25 (17955-17960).
Refs: 45
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Porphyromonas gingivalis** produces arginine-specific cysteine proteinase (Arg-gingipain, RGP) and lysine-specific cysteine proteinase (Lys-gingipain, KGP) in the extracellular and cell-associated forms. Two separate genes (rgpA and rgpB) and a single gene (kgp) have been found to encode RGP and KGP, respectively. We constructed rgpA rgpB kgp triple mutants by homologous recombination with cloned rgp and kgp DNA interrupted by drug resistance gene markers. The triple mutants showed no RGP or KGP activity in either cell extracts or culture supernatants. The culture supernatants of the triple mutants grown in a rich medium had no proteolytic activity toward bovine serum albumin or gelatin derived from human type I collagen. Moreover, the mutants did not grow in a defined medium containing bovine serum albumin as the sole carbon/energy source. These results indicate that the proteolytic activity of **P.**

gingivalis toward bovine serum albumin and gelatin derived from human type I collagen appears to be attributable to RGP and KGP. The **hemagglutinin** gene hagA of **P. gingivalis** possesses the adhesin domain regions responsible for hemagglutination and hemoglobin binding that are also located in the C-terminal regions of rgpA and kgp. A rgpA kgp hagA triple mutant constructed in this study exhibited no hemagglutination using sheep erythrocytes or hemoglobin binding activity, as determined by a solid-phase binding assay with horseradish peroxidase-conjugated human hemoglobin, indicating that the adhesin domains seem to be particularly important for **P. gingivalis** cells to agglutinate erythrocytes and bind hemoglobin, leading to heme acquisition.

L13 ANSWER 10 OF 32 MEDLINE
ACCESSION NUMBER: 1999369863 MEDLINE
DOCUMENT NUMBER: 99369863 PubMed ID: 10438761
TITLE: Hemoglobinase activity of the lysine gingipain protease (Kgp) of **Porphyromonas gingivalis** W83.
AUTHOR: Lewis J P; Dawson J A; Hannis J C; Muddiman D; Macrina F L
CORPORATE SOURCE: Institute of Oral and Craniofacial Molecular Biology, Virginia Commonwealth University, Richmond, Virginia 23298, USA.
CONTRACT NUMBER: DE04224 (NIDCR)
DE07606 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (1999 Aug) 181 (16) 4905-13.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990921
Last Updated on STN: 20000303
Entered Medline: 19990903

AB **Porphyromonas gingivalis**, an important periodontal disease pathogen, forms black-pigmented colonies on blood agar. Pigmentation is believed to result from accumulation of iron protoporphyrin IX (FePPIX) derived from erythrocytic hemoglobin. The Lys-X (Lys-gingipain) and Arg-X (Arg-gingipain) cysteine proteases of **P. gingivalis** bind and degrade erythrocytes. We have observed that mutations abolishing activity of the Lys-X-specific cysteine protease, Kgp, resulted in loss of black pigmentation of **P. gingivalis** W83. Because the hemagglutinating and hemolytic potentials of mutant strains were reduced but not eliminated, we hypothesized that this protease played a role in acquisition of FePPIX from hemoglobin. In contrast to Arg-gingipain, Lys-gingipain was not inhibited by hemin, suggesting that this protease played a role near the cell surface where high concentrations of hemin confer the black pigmentation. Human hemoglobin contains 11 Lys residues in the alpha chain and 10 Lys residues in the beta chain. In contrast, there are only three Arg residues in each of the alpha and beta chains. These observations are consistent with human hemoglobin being a preferred substrate for Lys-gingipain but not Arg-gingipain. The ability of the Lys-gingipain to cleave human hemoglobin at Lys residues was confirmed by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry of hemoglobin fragments resulting from digestion with the purified protease. We were able to detect several of the predicted hemoglobin fragments rendered by digestion with purified Lys-gingipain. Thus, we postulate that the Lys-gingipain of **P. gingivalis** is a hemoglobinase which plays a role in heme and iron

uptake by effecting the accumulation of FePPIX on the bacterial cell surface.

L13 ANSWER 11 OF 32 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999296589 MEDLINE
DOCUMENT NUMBER: 99296589 PubMed ID: 10368154
TITLE: Porphyrin-mediated binding to hemoglobin by the **HA2** domain of cysteine proteinases (gingipains) and **hemagglutinins** from the periodontal pathogen **Porphyromonas gingivalis.**
AUTHOR: DeCarlo A A; Paramaesvaran M; Yun P L; Collyer C; Hunter N
CORPORATE SOURCE: Institute of Dental Research, Sydney, Australia.. adecarlo@uab.edu
SOURCE: JOURNAL OF BACTERIOLOGY, (1999 Jun) 181 (12) 3784-91. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 20000303
Entered Medline: 19990715

AB Heme binding and uptake are considered fundamental to the growth and virulence of the gram-negative periodontal pathogen **Porphyromonas gingivalis**. We therefore examined the potential role of the dominant **P. gingivalis** cysteine proteinases (gingipains) in the acquisition of heme from the environment. A recombinant hemoglobin-binding domain that is conserved between two predominant gingipains (domain **HA2**) demonstrated tight binding to hemin (Kd = 16 nM), and binding was inhibited by iron-free protoporphyrin IX (Ki = 2.5 microM). Hemoglobin binding to the gingipains and the recombinant **HA2** (rHA2) domain (Kd = 2.1 nM) was also inhibited by protoporphyrin IX (Ki = 10 microM), demonstrating an essential interaction between the **HA2** domain and the heme moiety in hemoglobin binding. Binding of rHA2 with either hemin, protoporphyrin IX, or hematoporphyrin was abolished by establishing covalent linkage of the protoporphyrin propionic acid side chains to fixed amines, demonstrating specific and directed binding of rHA2 to these protoporphyrins. A monoclonal antibody which recognizes a peptide epitope within the **HA2** domain was employed to demonstrate that **HA2**-associated hemoglobin-binding activity was expressed and released by **P. gingivalis** cells in a batch culture, in parallel with proteinase activity. Cysteine proteinases from **P. gingivalis** appear to be multidomain proteins with functions for hemagglutination, erythrocyte lysis, proteolysis, and heme binding, as demonstrated here. Detailed understanding of the biochemical pathways for heme acquisition in **P. gingivalis** may allow precise targeting of this critical metabolic aspect for periodontal disease prevention.

L13 ANSWER 12 OF 32 MEDLINE
ACCESSION NUMBER: 1998362001 MEDLINE
DOCUMENT NUMBER: 98362001 PubMed ID: 9694880
TITLE: Involvement of a lysine-specific cysteine proteinase in hemoglobin adsorption and heme accumulation by **Porphyromonas gingivalis.**
AUTHOR: Okamoto K; Nakayama K; Kadowaki T; Abe N; Ratnayake D B; Yamamoto K
CORPORATE SOURCE: Department of Pharmacology, Kyushu University Faculty of

Dentistry, Higashi-ku, Fukuoka 812-8582, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Aug 14) 273 (33) 21225-31.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19980925
Last Updated on STN: 20000303
Entered Medline: 19980914

AB The oral anaerobic bacterium **Porphyromonas gingivalis**, a major pathogen of advanced adult periodontitis, produces a novel class of cysteine proteinases in both cell-associated and secretory forms. A lysine-specific cysteine proteinase (Lys-gingipain, KGP), as well as an arginine-specific cysteine proteinase (Arg-gingipain), is a major trypsin-like proteinase of the organism. Recent studies indicate that the secreted KGP is implicated in the destruction of periodontal tissue and the disruption of host defense mechanisms. In this study, we have constructed a KGP-deficient mutant to determine whether the cell-associated KGP is important for pathophysiology of the organism. Although the mutant retained the strong ability to disrupt the bactericidal activity of polymorphonuclear leukocytes, its hemagglutination activity was reduced to about one-half that observed with the wild-type strain. More important, the mutant did not form black-pigmented colonies on blood agar plates, indicating the defect of hemoglobin adsorption and heme accumulation. Immunoblot analysis showed that the expression of a 19-kDa hemoglobin receptor protein, which is thought to be responsible for hemoglobin binding by the organism, was greatly retarded in this mutant. The mutant also showed a marked decrease in the ability to degrade fibrinogen. These results suggest the possible involvement of KGP in the hemoglobin binding and heme accumulation of the organism and in the bleeding tendency in periodontal pockets.

L13 ANSWER 13 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:55152 BIOSIS
DOCUMENT NUMBER: PREV199900055152
TITLE: Life below the gum line: Pathogenic mechanisms of **Porphyromonas gingivalis**.
AUTHOR(S): Lamont, Richard J. (1); Jenkinson, Howard F.
CORPORATE SOURCE: (1) Dep. Oral Biology, Box 357132, University Washington, Seattle, WA 98195-7132 USA
SOURCE: Microbiology and Molecular Biology Reviews, (Dec., 1998) Vol. 62, No. 4, pp. 1244-1263.
ISSN: 1092-2172.
DOCUMENT TYPE: General Review
LANGUAGE: English

L13 ANSWER 14 OF 32 MEDLINE
ACCESSION NUMBER: 1999138114 MEDLINE
DOCUMENT NUMBER: 99138114 PubMed ID: 9972167
TITLE: The **porphyromonas gingivalis** prtP/kgp homologue exists as two open reading frames in strain 381.
AUTHOR: Han N; Lepine G; Whitlock J; Wojciechowski L; Progulske-Fox A
CORPORATE SOURCE: Department of Oral Biology, University of Florida, Gainesville 32610-0424, USA.
CONTRACT NUMBER: DE 00336 (NIDCR)
DE 07496 (NIDCR)

SOURCE: ORAL DISEASES, (1998 Sep) 4 (3) 170-9.
Journal code: 9508565. ISSN: 1354-523X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
OTHER SOURCE: GENBANK-U68468
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990301
Last Updated on STN: 20030331
Entered Medline: 19990218

AB **P. gingivalis** is considered to be a major pathogen of adult periodontitis. Among its cadre of putative virulence factors are **hemagglutinins** (adhesins) and proteases. We here report the cloning, sequencing and characterization of two genes, designated kgp(381) and hagD. Kgp(381), an open reading frame (ORF) of 1095 bp encoding a 40.1 kda protein, has high homology to the proteolytic domain of cysteine protease/**hemagglutinin** genes. HagD, an ORF of 4077 bp encoding a 147.1 kda protein, contains one HArep sequence which establishes it as an additional member of the HArep multigene family. Although similar in sequence to kgp and prtP which were identified from strains HG66 and W12, respectively, the kgp(381)-hagD genes have several characteristics which distinguish them from kgp and prtP. Foremost among these is a single base difference which produces a termination codon and an immediate frame shift resulting in two ORFs in strain 381 as compared to one ORF in strains HG66 and W12. In addition, a 172 amino acid sequence near the C-terminal end of hagD has very low identity (20.5-27.8%) to the corresponding region of kgp and prtP. These demonstrate that the homologue of kgp and prtP in strain 381 occurs as two separate genes which may genetically separate the adhesive and enzymatic domains of Kgp and PrtP proteins. Reverse polymerase chain reaction (PCR) analysis indicates that hagD expression is regulated by hemin concentration.

L13 ANSWER 15 OF 32 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998087560 MEDLINE
DOCUMENT NUMBER: 98087560 PubMed ID: 9425248
TITLE: Hemin regulation of hemoglobin binding by **Porphyromonas gingivalis**.
AUTHOR: Smalley J W; Birss A J; McKee A S; Marsh P D
CORPORATE SOURCE: Unit of Oral Biology, Department of Clinical Dental Sciences, The University of Liverpool, Liverpool L69 3BX, UK.
SOURCE: CURRENT MICROBIOLOGY, (1998 Feb) 36 (2) 102-6.
Journal code: 7808448. ISSN: 0343-8651.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980319
Last Updated on STN: 19980319
Entered Medline: 19980310

AB Hemoglobin binding to chemostat-grown hemin-excess and hemin-limited cells of **Porphyromonas gingivalis** W50, and to cells of the avirulent, beige-pigmenting variant W50/BE1, was quantified. Hemin-excess W50 bound more hemoglobin than hemin-limited W50, mirroring the **hemin-binding** ability of these cells [Microb Ecol Health Dis 7:9-15, 1994]. In contrast to hemin, hemoglobin binding was not enhanced by sodium dithionite. The hemoglobin-binding capacity of hemin-excess W50/BE1 was below that of hemin-limited W50 and only observed

under oxidizing conditions. Scatchard analysis revealed similar affinity constants for hemin-excess and hemin-limited W50, and confirmed a lower binding maximum for the latter. Hemin-excess W50/BE1 displayed cooperative binding of hemoglobin. These differences in binding were reflected in the binding of a horse radish peroxidase-conjugated hemoglobin (HHRPO) in a dot-blot assay. However, neither the 32-kDa **hemin-binding** protein, nor its 19-kDa heat-modified form, from either hemin-limited W50 or hemin-excess W50/BE1, bound this conjugate. These data indicate that hemoglobin binding by **P. gingivalis** is hemin-regulated and occurs via a mechanism different from **hemin binding**.

L13 ANSWER 16 OF 32 MEDLINE
ACCESSION NUMBER: 97386416 MEDLINE
DOCUMENT NUMBER: 97386416 PubMed ID: 9244265
TITLE: The Tla protein of **Porphyromonas gingivalis** W50: a homolog of the RI protease precursor (PrpRI) is an outer membrane receptor required for growth on low levels of hemin.
AUTHOR: Aduse-Opoku J; Slaney J M; Rangarajan M; Muir J; Young K A; Curtis M A
CORPORATE SOURCE: Department of Oral Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, England.. J.Aduse@mds.qmw.ac.uk
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Aug) 179 (15) 4778-88. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-P27772; GENBANK-U00007; GENBANK-U59691; GENBANK-V56084; GENBANK-X77924; GENBANK-Y07618
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 20000303
Entered Medline: 19971023

AB The prpR1 gene of **Porphyromonas gingivalis** W50 encodes the polyprotein precursor (PrpRI) of an extracellular arginine-specific protease. PrpRI is organized into four distinct domains (pro, alpha, beta, and gamma) and is processed to a heterodimeric protease (RI) which comprises the alpha and beta components in a noncovalent association. The alpha component contains the protease active site, whereas the beta component appears to have a role in adherence and hemagglutination processes. DNA sequences homologous to the coding region for the RI beta component are present at multiple loci on the **P. gingivalis** chromosome and may represent a family of related genes. In this report, we describe the cloning, sequence analysis, and characterization of one of these homologous loci isolated in plasmid pJM7. The 6,041-bp **P. gingivalis** DNA fragment in pJM7 contains a major open reading frame of 3,291 bp with coding potential for a protein with an Mr 118,700. An internal region of the deduced sequence (V304 to N768) shows 98% identity to the beta domain of PrpRI, and the recombinant product of pJM7 is immunoreactive with an antibody specific to the RI beta component. The N terminus of the deduced sequence has regional similarity to TonB-linked receptors which are frequently involved in periplasmic translocation of hemin, iron, colicins, or vitamin B12 in other bacteria. We have therefore designated this gene tla (TonB-linked adhesin). In contrast to the parent strain, an isogenic mutant of **P. gingivalis** W50 in which the tla was insertionally inactivated was unable to grow in medium containing low concentrations of

hemin (<2.5 mg liter(-1)), and hemin-depleted cells of this mutant failed to respond to hemin in an agar diffusion plate assay. These data suggest a role for this gene product in hemin acquisition and utilization. Furthermore, the mutant produced significantly less arginine- and lysine-specific protease activities than the parent strain, indicating that there may be a regulatory relationship between tla and other members of this gene family.

L13 ANSWER 17 OF 32 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97158652 MEDLINE
DOCUMENT NUMBER: 97158652 PubMed ID: 9006012
TITLE: Detection and comparison of specific **hemin binding** by **Porphyromonas gingivalis** and Prevotella intermedia.
AUTHOR: Tompkins G R; Wood D P; Birchmeier K R
CORPORATE SOURCE: Department of Oral Biology, School of Dentistry, Medical College of Georgia, Augusta 30912-1126, USA.. gtompkin@mail.mcg.edu
CONTRACT NUMBER: DE10272 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (3) 620-6. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970313
Last Updated on STN: 20000303
Entered Medline: 19970228

AB A radioligand assay was designed to detect and compare specific **hemin binding** by the periodontal anaerobic black-pigmenting bacteria (BPB) **Porphyromonas gingivalis** and Prevotella intermedia. The assay included physiological concentrations of the **hemin-binding** protein rabbit serum albumin (RSA) to prevent self-aggregation and nonspecific interaction of hemin with cellular components. Under these conditions, heme-starved P. intermedia cells (two strains) expressed a single binding site species (4,100 to 4,600 sites/cell) with a dissociation constant (Kd) of 1.0 x 10(-9) M. Heme-starved **P. gingivalis** cells (two strains) expressed two binding site species; the higher-affinity site (1,000 to 1,500 sites/cell) displayed a Kd of between 3.6 x 10(-11) and 9.6 x 10(-11) M, whereas the estimated Kd of the lower-affinity site (1.9 x 10(5) to 6.3 x 10(5) sites/cell) ranged between 2.6 x 10(-7) and 6.5 x 10(-8) M. Specific binding was greatly diminished in heme-replete cells of either BPB species and was not displayed by iron-replete Escherichia coli cells, which bound as much hemin in the absence of RSA as did P. intermedia. **Hemin binding** by BPB was reduced following treatment with protein-modifying agents (heat, pronase, and N-bromosuccinimide) and was blocked by protoporphyrin IX and hemoglobin but not by Congo red. Hemopexin also inhibited bacterial **hemin binding**. These findings indicate that both **P. gingivalis** and P. intermedia express heme-repressible proteinaceous **hemin-binding** sites with affinities intermediate between those of serum albumin and hemopexin. **P. gingivalis** exhibited a 10-fold-greater specific binding affinity and greater heme storage capacity than did P. intermedia, suggesting that the former would be ecologically advantaged with respect to heme acquisition.

L13 ANSWER 18 OF 32 MEDLINE DUPLICATE 7

ACCESSION NUMBER: 96239049 MEDLINE
DOCUMENT NUMBER: 96239049 PubMed ID: 8675338
TITLE: Hemin-induced modifications of the antigenicity and **hemin-binding** capacity of **Porphyromonas gingivalis** lipopolysaccharide.
AUTHOR: Cutler C W; Eke P I; Genco C A; Van Dyke T E; Arnold R R
CORPORATE SOURCE: Department of Biomedical Sciences and Periodontics, Baylor College of Dentistry, Dallas, Texas, USA.
CONTRACT NUMBER: DE00214 (NIDCR)
DE09161 (NIDCR)
RR03034 (NCRR)
SOURCE: INFECTION AND IMMUNITY, (1996 Jun) 64 (6) 2282-7.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960822
Last Updated on STN: 20000303
Entered Medline: 19960812

AB Previous studies have shown that the physical, biochemical, and antigenic properties of the bacterial outer membrane are profoundly influenced by the growth environment. In the present study, the effects of growth in hemin-replete (H+) and hemin-depleted (H-) media on the lipopolysaccharide (LPS) of the oral pathogen **Porphyromonas gingivalis** were investigated. Our studies show that LPS from **P. gingivalis** cultured in H+ media (H+LPS) expressed additional low-molecular-mass antigens, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot (immunoblot) analysis. Particularly evident was a 26-kDa antigen (26 LPSC) that was lost from the LPS upon transfer of **P. gingivalis** into H- media. The loss of the 26 LPSC was accompanied by a marked reduction in the **hemin-binding** capacity of the LPS. The 26 LPSC was refractory to Coomassie blue staining and proteinase K digestion. H+LPS from strain W50/BE1, a nonpigmented pleiotropic strain, lacked the 26 LPSC and did not bind hemin. Polyclonal antiserum raised to whole-cell antigens of **P. gingivalis** A7436, W83, and HG405 grown in H+ media, but not in H- media, recognized the 26 LPSC in the purified H+LPS from any of the three strains. The immunoreactivities of sera from humans with (n = 24) or without (n = 25) periodontitis to the 26 LPSC and other H+LPS determinants were analyzed by Western blot. Overall, 75% of adult periodontitis patient sera reacted with multiple bands in the H+LPS stepladder, particularly in the range of 14 to 27 kDa. In contrast, only 20% of control sera reacted faintly with H+LPS bands in the range 27 to 34 kDa. The 26 LPSC was recognized by over 40% of sera from adult patients with periodontitis and none of the healthy control sera. Taken together, these results suggest that the antigenicity and **hemin-binding** properties of **P. gingivalis** LPS can be modified by growth in H+ media.

L13 ANSWER 19 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:259689 BIOSIS
DOCUMENT NUMBER: PREV199698815818
TITLE: Comparison of specific **hemin-binding** by **Porphyromonas gingivalis** and Prevotella intermedia.
AUTHOR(S): Tompkins, G. R.
CORPORATE SOURCE: Med. Coll. Georgia, Augusta, GA USA

SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1996) Vol. 96, No. 0, pp. 227. Meeting Info.: 96th General Meeting of the American Society for Microbiology New Orleans, Louisiana, USA May 19-23, 1996
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L13 ANSWER 20 OF 32 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 96425257 MEDLINE
DOCUMENT NUMBER: 96425257 PubMed ID: 8827708
TITLE: Isolation and characterization of a **hemin-binding** cell envelope protein from **Porphyromonas gingivalis**.
AUTHOR: Kim S J; Chu L; Holt S C
CORPORATE SOURCE: Department of Microbiology, University of Texas Health Center at San Antonio 78284, USA.
CONTRACT NUMBER: DE-07627 (NIDCR)
SOURCE: MICROBIAL PATHOGENESIS, (1996 Jul) 21 (1) 65-70.
Journal code: 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20000303
Entered Medline: 19961209

AB A 30 kDa (heated 24 kDa) **hemin-binding** protein whose expression is both hemin and iron regulated was identified and purified in **Porphyromonas gingivalis** 381. A strong **hemin-binding** function was found by LDS-PAGE and TMBZ staining when cells were grown under hemin (iron)-limited conditions. N-terminal amino acid sequence analysis of CNBr-digested 24 kDa **hemin binding** protein revealed that this protein belongs to a new, so far undescribed **hemin-binding** class of proteins.

L13 ANSWER 21 OF 32 MEDLINE
ACCESSION NUMBER: 97096936 MEDLINE
DOCUMENT NUMBER: 97096936 PubMed ID: 8941757
TITLE: Duplication and differential expression of **hemagglutinin** genes in **Porphyromonas gingivalis**.
AUTHOR: Lepine G; Progulske-Fox A
CORPORATE SOURCE: Department of Oral Biology, College of Dentistry, University of Florida, Gainesville, USA.
CONTRACT NUMBER: DE07496 (NIDCR)
SOURCE: ORAL MICROBIOLOGY AND IMMUNOLOGY, (1996 Apr) 11 (2) 65-78.
Journal code: 8707451. ISSN: 0902-0055.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Dental Journals
OTHER SOURCE: GENBANK-Z27394
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 20030318
Entered Medline: 19970114

AB A third **hemagglutinin** gene, defined as hagC, was cloned from

Porphyromonas gingivalis 381 and sequenced. This gene was found to encode a protein highly homologous (98.6%) to the previously reported HagB **hemagglutinin** protein. The upstream and downstream regions of hagB and hagC were found to share less than 40% homology compared with 99% for their open reading frames. The antigenic relationship between the two **hemagglutinins** was demonstrated by Western blot analysis. When expressed in an in vitro transcription-translation system, both genes encoded a protein with a molecular mass of 49 kDa. As determined by reverse transcription polymerase chain reaction, the steady-state levels of hagB and hagC mRNAs were found to vary according to the growth phase and hemin concentration. The amount of transcripts decreased in hemin-limited conditions or in the absence of hemin. Furthermore, hagB mRNAs were detected in the early logarithmic growth phase compared with the hagC transcripts, which were detected only in the mid-exponential phase of growth.

L13 ANSWER 22 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:382086 BIOSIS
DOCUMENT NUMBER: PREV199699104442
TITLE: Haemin binding as a factor in the virulence of **Porphyromonas gingivalis**.
AUTHOR(S): Smalley, John W. (1); Birss, Andrew J.; McKee, Ailsa S.; Marsh, Philip D.
CORPORATE SOURCE: (1) Unit Oral Biol., Edwards Build., Dep. Clin. Dent. Sci., Univ. Liverpool, Liverpool L69 3BX UK
SOURCE: FEMS Microbiology Letters, (1996) Vol. 141, No. 1, pp. 65-70.
ISSN: 0378-1097.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Haemin (iron protoporphyrin IX) is an essential growth factor for the periodontal pathogen, **Porphyromonas gingivalis**. Iron protoporphyrin IX (IPP IX) binding to the avirulent **P. gingivalis** beige variant (W50/BE1) and the black-pigmenting parent wild-type strain W50 was quantified. W50/BE1 grown in a chemostat under haemin excess-bound IPP IX under both oxidising and reducing conditions but with both lower capacity and avidity than either the haemin-limited- and haemin-excess-grown parent strain W50. Rosenthal plots for W50/BE1 indicated cooperative binding. W50/BE1 cells expressed a 32 kDa outer membrane haemin-binding protein when grown under conditions of haemin excess, and this strain might serve as a useful source from which to isolate this protein. The reduced IPP IX binding ability of W50/BE1 may be the rate-limiting factor for haem uptake and explain the reduced virulence and slower rate of pigmentation of this strain.

L13 ANSWER 23 OF 32 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 95309997 MEDLINE
DOCUMENT NUMBER: 95309997 PubMed ID: 7790057
TITLE: Characterization of a Tn4351-generated hemin uptake mutant of **Porphyromonas gingivalis**: evidence for the coordinate regulation of virulence factors by hemin.
AUTHOR: Genco C A; Simpson W; Forng R Y; Egal M; Odusanya B M
CORPORATE SOURCE: Department of Microbiology and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310-1495, USA.
CONTRACT NUMBER: DE09161 (NIDCR)
RR03034 (NCRR)
SOURCE: INFECTION AND IMMUNITY, (1995 Jul) 63 (7) 2459-66.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950807
Last Updated on STN: 20000303
Entered Medline: 19950727

AB The ability of **Porphyromonas gingivalis** to acquire iron in the iron-limited environment of the host is crucial to the colonization of this organism. We report here on the isolation and characterization of a transpositional insertion mutant of **P. gingivalis** A7436 (designated MSM-3) which is defective in the utilization and transport of hemin. **P. gingivalis** MSM-3 was selected on the basis of its nonpigmented phenotype on anaerobic blood agar following mutagenesis with the Bacteroides fragilis transposon Tn4351. **P. gingivalis** MSM-3 grew poorly when supplied with hemin as a sole source of iron; however, growth was observed with hemoglobin or inorganic iron. **P. gingivalis** MSM-3 grown in either hemin-replete or hemin-depleted conditions bound and transported less [14C]hemin or [59Fe]hemin than did the parent strain. At 4 h, **P. gingivalis** MSM-3 grown in hemin-replete conditions transported only 10,000 pmol of hemin per mg of protein, or 14% of the amount transported by **P. gingivalis** A7436. Unlike **P. gingivalis** A7436, **hemin binding** and transport by **P. gingivalis** MSM-3 were not tightly regulated by hemin or iron. Examination of **P. gingivalis** MSM-3 cultures by electron microscopy revealed an overproduction of membrane vesicles, and determination of the dry weight of purified vesicles indicated that **P. gingivalis** MSM-3 produced twice as much membrane vesicles as did strain A7436. Extracellular vesicles isolated from **P. gingivalis** MSM-3 also were found to express increased hemolytic and trypsin-like protease activities compared with the parent strain. When inoculated into subcutaneous chambers implanted in mice, **P. gingivalis** MSM-3 was highly infectious and more invasive than the parent strain, as indicated by secondary lesion formation and death. Taken together, these results indicate that the decreased transport of hemin by **P. gingivalis** MSM-3 results in the increased expression of several virulence factors which may be coordinately regulated by hemin.

L13 ANSWER 24 OF 32 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 94274305 MEDLINE
DOCUMENT NUMBER: 94274305 PubMed ID: 8005678
TITLE: Binding and accumulation of hemin in **Porphyromonas gingivalis** are induced by hemin.
AUTHOR: Genco C A; Odusanya B M; Brown G
CORPORATE SOURCE: Department of Microbiology and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310.
CONTRACT NUMBER: DE09161 (NIDCR)
RR03034 (NCRR)
SOURCE: INFECTION AND IMMUNITY, (1994 Jul) 62 (7) 2885-92.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940729
Last Updated on STN: 20000303
Entered Medline: 19940720

AB Although hemin is an essential nutrient for the black-pigmented oral bacterium **Porphyromonas gingivalis**, the mechanisms involved in **hemin binding** and uptake are poorly defined. In this study, we have examined the binding of hemin and Congo red (CR) to **P. gingivalis** whole cells and have defined the conditions for maximal binding. Additionally, the accumulation of hemin by **P. gingivalis** under growing conditions has been characterized. **P. gingivalis** A7436 was grown under hemin- or iron-deplete conditions (basal medium [BM] or Schaedler broth with dipyridyl [SBD]) or under hemin- or iron-replete conditions (BM with hemin [BMH] or Schaedler broth [SB]), and hemin and CR binding were assessed spectrophotometrically. Binding of hemin by **P. gingivalis** whole cells was rapid and was observed in samples obtained from cells grown under hemin- and iron-replete and hemin-deplete conditions but was not observed in cells grown under iron limitation. We also found that **P. gingivalis** whole cells bound more hemin when grown in BMH or SB than cells grown in BM or SBD. Binding of CR by **P. gingivalis** A7436 was also enhanced when cells were grown in the presence of hemin or when cells were incubated with hemin prior to CR binding. **Hemin binding** and accumulation were also assessed using [14C]hemin and [59Fe]hemin under growing conditions. Both [14C]hemin and [59Fe]hemin were accumulated by **P. gingivalis**, indicating that iron and the porphyrin ring were taken into the cell. Binding and accumulation of hemin under growing conditions were also induced by growth of **P. gingivalis** in hemin-replete media. Hemin accumulation was inhibited by the addition of KCN to **P. gingivalis** cultures, indicating that active transport was required for hemin uptake. [14C]**hemin binding** and accumulation were also inhibited by the addition of either cold hemin or protoporphyrin IX. Taken together, these results indicate that **P. gingivalis** transports the entire hemin moiety into the cell and that the binding and accumulation of hemin are induced by growth of cultures in the presence of hemin.

L13 ANSWER 25 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1994:330723 BIOSIS
DOCUMENT NUMBER: PREV199497343723
TITLE: Cloning and characterization of a fourth putative **hemagglutinin** gene from **Porphyromonas gingivalis.**
AUTHOR(S): Lepine, G.; Whitlock, J. A.; Han, N.; Progulskefox, A.
CORPORATE SOURCE: Univ. Florida, Gainesville, FL USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1994) Vol. 94, No. 0, pp. 116. Meeting Info.: 94th General Meeting of the American Society for Microbiology Las Vegas, Nevada, USA May 23-27, 1994 ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English

L13 ANSWER 26 OF 32 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 95095027 MEDLINE
DOCUMENT NUMBER: 95095027 PubMed ID: 8001768
TITLE: Transposon-induced pigment-deficient mutants of **Porphyromonas gingivalis.**
AUTHOR: Hoover C I; Yoshimura F
CORPORATE SOURCE: Department of Microbiology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan.
SOURCE: FEMS MICROBIOLOGY LETTERS, (1994 Nov 15) 124 (1) 43-8.

Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19990129
Entered Medline: 19950126

AB Trypsin-like protease activity, hemagglutination activity, and accumulation of heme-containing compounds (black pigment) are considered to be virulence factors of **Porphyromonas gingivalis**. Transposon-mutagenesis was used for the first time to isolate pigment-deficient mutants. These mutants exhibited simultaneous deficiency in trypsin-like protease activity and hemagglutination activity. Two major membrane-associated proteins, observed by SDS-PAGE with the parent strain, were essentially absent from the mutant strains. Immunoblot analysis indicated that these two proteins correspond to putative **hemagglutinin** and **hemagglutinin**/protease products of **P. gingivalis**. Each mutant contained only one transposon insertion, thus the pleiotropic phenotype resulted from single site-specific mutations. The results indicate that trypsin-like protease activity is required for accumulation of protoheme from hemoglobin by **P. gingivalis** and that genetic and/or physiological linkage exists between trypsin-like protease activity and hemagglutination activity.

L13 ANSWER 27 OF 32 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 94042917 MEDLINE
DOCUMENT NUMBER: 94042917 PubMed ID: 8226688
TITLE: Hemin uptake in **Porphyromonas gingivalis** : Omp26 is a **hemin-binding** surface protein.
AUTHOR: Bramanti T E; Holt S C
CORPORATE SOURCE: Department of Periodontics, University of Texas Health Science Center at San Antonio 78284-7894.
CONTRACT NUMBER: DE00152 (NIDCR)
DE07267 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Nov) 175 (22) 7413-20.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 20000303
Entered Medline: 19931214

AB A 26-kDa outer membrane protein (Omp26) has been proposed to play a role in hemin acquisition by **Porphyromonas gingivalis** (T. E. Bramanti and S. C. Holt, J. Bacteriol. 174:5827-5839, 1992). We studied [55Fe]hemin uptake in **P. gingivalis** grown under conditions of hemin starvation (Omp26 expressed on the outer membrane surface) and hemin excess (Omp26 not expressed on surface). [55Fe]hemin uptake occurred rapidly in hemin-starved cells which incorporated up to 70% of total [55Fe]hemin within 3 min. **P. gingivalis** grown under hemin-starved conditions or treated with the iron chelator 2,2'-bipyridyl to induce an iron stress took up six times more [55Fe]hemin than hemin-excess-grown cells. Polyclonal monospecific anti-Omp26 antibody added to hemin-starved cells inhibited

[55Fe]hemin uptake by more than 50%, whereas preimmune serum had no effect. [55Fe]hemin uptake in hemin-starved **P. gingivalis** was inhibited (36 to 67%) in the presence of equimolar amounts of unlabeled hemin, protoporphyrin IX, zinz protoporphyrin, and Congo red dye but was not inhibited in the presence of non-hemin-containing iron sources. Heat shock treatment (45 degrees C) of hemin-excess-grown **P. gingivalis** (which cases translocation of Omp26 to the surface) increased [55Fe]hemin uptake by threefold after 3 min in comparison with cells grown at 37 degrees C.(ABSTRACT TRUNCATED AT 250 WORDS)

L13 ANSWER 28 OF 32 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 92394885 MEDLINE
DOCUMENT NUMBER: 92394885 PubMed ID: 1522061
TITLE: Localization of a **Porphyromonas gingivalis** 26-kilodalton heat-modifiable, hemin-regulated surface protein which translocates across the outer membrane.
AUTHOR: Bramanti T E; Holt S C
CORPORATE SOURCE: Department of Periodontics, University of Texas Health Science Center, San Antonio 78284-7894.
CONTRACT NUMBER: DE00152 (NIDCR)
DE07267 (NIDCR)
SOURCE: JOURNAL OF BACTERIOLOGY, (1992 Sep) 174 (18) 5827-39.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921023
Last Updated on STN: 20000303
Entered Medline: 19921013

AB We recently identified a 26-kDa hemin-repressible outer membrane protein (Omp26) expressed by the periodontal pathogen **Porphyromonas gingivalis**. We report the localization of Omp26, which may function as a component of a hemin transport system in **P. gingivalis**. Under hemin-deprived conditions, **P. gingivalis** expressed Omp26, which was then lost from the surface after a shift back into hemin-rich conditions. Experiments with 125I labeling of surface proteins to examine the kinetics of mobilization of Omp26 determined that it was rapidly (within less than 1 min) lost from the cell surface after transfer into a hemin-excess environment. When cells grown under conditions of hemin excess were treated with the iron chelator 2,2'-bipyridyl, Omp26 was detected on the cell surface after 60 min. One- and two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analyses using purified anti-Omp26 monospecific polyclonal immunoglobulin G antisera established that Omp26 was heat modifiable (39 kDa unheated) and consisted of a single protein species. Immunogold labeling of negatively stained and chemically fixed thin-section specimens indicated that Omp26 was associated with the cell surface and outer leaflet of the **P. gingivalis** outer membrane in hemin-deprived conditions but was buried in the deeper recesses of the outer membrane in hemin-excess conditions. Analysis of subcellular fractions of **P. gingivalis** grown either in hemin-excess or hemin-deprived conditions detected Omp26 only in the cell envelope fraction, not in the cytoplasmic fraction or culture supernatant. Limited proteolytic digestion of hemin-deprived **P. gingivalis** with trypsin and proteinase K verified the surface location of Omp26 as well as its susceptibility to proteolytic digestion.

Heat shock treatment of hemin-excess-grown **P. gingivalis** also resulted in Omp26 translocation onto the outer membrane surface even in the presence of hemin. Furthermore, hemin repletion of heat-shocked, hemin-deprived **P. gingivalis** did not result in Omp26 translocation off the outer membrane surface, suggesting that thermal stress inactivates this transmembrane event. This newly described outer membrane protein appears to be associated primarily with the outer membrane, in which it is exported to the outer membrane surface for **hemin binding** and may be imported across the outer membrane for intracellular hemin transport.

L13 ANSWER 29 OF 32 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1992:205097 BIOSIS
DOCUMENT NUMBER: BR42:98172
TITLE: CHARACTERIZATION AND LOCALIZATION OF THE 26 KDA PORPHYRIN **HEMIN BINDING** PROTEIN OF **PORPHYROMONAS-GINGIVALIS**.
AUTHOR(S): BRAMANTI T E; HOLT S C
CORPORATE SOURCE: UTHSC, SAN ANTONIO, TEXAS.
SOURCE: 21ST ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR DENTAL RESEARCH, BOSTON, MASSACHUSETTS, USA, MARCH 11-14, 1992. J DENT RES, (1992) 71 (SPEC ISSUE MAR), 145.
CODEN: JDREAF. ISSN: 0022-0345.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L13 ANSWER 30 OF 32 MEDLINE
ACCESSION NUMBER: 91169240 MEDLINE
DOCUMENT NUMBER: 91169240 PubMed ID: 2004696
TITLE: **Hemin-binding** property of **Porphyromonas gingivalis** outer membranes.
AUTHOR: Grenier D
CORPORATE SOURCE: Departement de Sante Buccale, Faculte de Medecine Dentaire, Universite de Montreal, Quebec, Canada.
SOURCE: FEMS MICROBIOLOGY LETTERS, (1991 Jan 1) 61 (1) 45-9.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910512
Last Updated on STN: 19910512
Entered Medline: 19910423
AB The present study indicates that the outer membranes of **Porphyromonas gingivalis** have a high affinity for hemin, an essential growth factor for this suspected periodontal pathogen. The **hemin-binding** property appears to be mediated by the lipopolysaccharides, particularly the lipid A region. It is suggested that this binding mechanism may be important for the growth and establishment of **P. gingivalis** in environments with a low hemin content such as periodontal pockets.

L13 ANSWER 31 OF 32 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 91012041 EMBASE
DOCUMENT NUMBER: 1991012041
TITLE: **Hemin-binding** property of **Porphyromonas gingivalis** outer membranes.
AUTHOR: Grenier D.

CORPORATE SOURCE: Dept. de Sante Buccale, Faculte de Medecine Dentaire, Universite de Montreal,Montreal, Que. H3C 3J7, Canada
SOURCE: FEMS Microbiology Letters, (1991) 77/1 (45-49). ISSN: 0378-1097 CODEN: FMLED7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The present study indicates that the outer membranes of **Porphyromonas gingivalis** have a high affinity for hemin, an essential growth factor for this suspected periodontal pathogen. The **hemin-binding** property appears to be mediated by the lipopolysaccharides, particularly the lipid A region. It is suggested that this binding mechanism may be important for the growth and establishment of **P. gingivalis** in environments with a low hemin content such as periodontal pockets.

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TITLE: Hemin levels in culture medium of Porphyromonas (Bacteroides) gingivalis regulate both **hemin binding** and trypsinlike protease production.
AUTHOR: Carman R J; Ramakrishnan M D; Harper F H
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AB Washed cells and Sarkosyl-insoluble outer membrane preparations of the black-pigmented bacteroides **Porphyromonas gingivalis** W50 bound hemin. The amount of hemin removed from a buffered solution by both cells and outer membranes was significantly larger if bacteria had been grown in broths supplemented with 5 mg of hemin per liter rather than none. Conversely, cells grown without supplemental hemin bound relatively little. However, all preparations bound some hemin. In addition, hemin regulated the production of significantly higher levels of trypsinlike protease by **P. gingivalis** W50. The nonpigmented variant, W50 BE1, showed no such responses to the levels of hemin in the growth medium.